# Trends in antimicrobial resistance in bloodstream infection isolates at a large urban hospital in Malawi (1998-2016): a surveillance study

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**Running head (short title):** Trends in BSI and AMR in Malawi

**Key words:** *E. coli*, *Klebsiella*, *S. pneumoniae,* Africa, ESBL, fluoroquinolone resistance.

## ABSTRACT

**Introduction** Bacterial bloodstream infection (BSI) is a common cause of morbidity and mortality in sub-Saharan Africa yet few facilities are able to conduct long-term surveillance. The Malawi-Liverpool-Wellcome Trust Clinical Research Programme has conducted sentinel surveillance of bacteraemia since 1998. We report long-term trends in BSI and antimicrobial resistance (AMR) from this surveillance.

**Methods** Blood cultures were routinely taken from adult and paediatric medical patients admitted to QECH with fever or suspicion of sepsis since 1998. Antimicrobial susceptibility tests were performed by the disc diffusion method according to BSAC guidelines. We analysed these data to describe trends in BSI and AMR from 1998-2016.

**Findings** 29,183 pathogens were isolated from 194,539 blood cultures. There was a significant decline in pathogen detection from 327·1/100,000/year to 120·2/100,000/year in 2016 ($p<0·0001$). 13,366/26,174 (51·1%) bacterial isolates were resistant to the Malawian first-line agents amoxicillin/penicillin, chloramphenicol and cotrimoxazole; 68·3% amongst Gram-negative and 6·6% of Gram-positive pathogens. The proportions of non-*Salmonellae* Enterobacteriaceae with ESBL or fluoroquinolone resistance rose significantly after 2003 to 61·9% in 2016 ($p<0·0001$). In contrast, over 92·0% of common Gram-positive pathogens remain susceptible to either penicillin or chloramphenicol. Methicillin resistant *S. aureus* (MRSA) was first reported in 1998 and represented 18·4% of *S. aureus* isolates in 2016.

**Interpretation** The rapid expansion of ESBL and fluoroquinolone resistance amongst common Gram-negative pathogens and emergence of MRSA highlight the growing challenge of BSIs that are effectively impossible to treat in this resource-limited setting.



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## INTRODUCTION

Bloodstream infection (BSI) is a leading cause of morbidity and mortality in both adults and children in sub-Saharan Africa (SSA).[1] In this region, the high burden of bacterial BSI has been strongly associated with the high prevalence of human immunodeficiency virus (HIV), malaria and malnutrition.[1-4] The clinical impact of BSI in SSA is exacerbated by inadequacy of diagnostic facilities, precluding both timely diagnosis of severe bacterial infection and implementation of appropriate antimicrobial therapy.[5]

Sentinel bacteraemia surveillance has been conducted at Queen Elizabeth Central Hospital (QECH), Blantyre, Malawi, a setting with high prevalence of HIV, malaria and malnutrition since 1998[6] and Nontyphoidal *Salmonellae* (NTS), *S.* Typhi and *Streptococcus pneumoniae* were previously identified as leading causes of BSI.[6-8] Widespread multidrug resistant (MDR) NTS necessitated the introduction and increasingly extensive use of ciprofloxacin (2002) and ceftriaxone (2004) for the empirical management of sepsis.[7,9] Extended spectrum beta-lactamase (ESBL) producing and fluoroquinolone resistant (FQR) Enterobacteriaceae have been reported in different settings around the globe where cephalosporins and fluoroquinolones have been in use.[10-14] This includes Blantyre, where ESBL-producing and FQR *E. coli*, *Klebsiella and Salmonellae* isolates were previously identified,[15-17] however, the full burden of ESBL and FQR among the Gram-negative pathogens in this setting has yet to be described.

In contrast, among Gram-positive pathogens we have previously reported fluctuating levels of pneumococcal resistance to penicillin ranging from 9·0-18·0%,[8] as has also been reported in other African settings.[18] Few studies in SSA have described the prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) and although in Malawi the geographical ubiquity of MRSA has increased, its prevalence remains unknown.[19]

Both long-term bacteraemia and AMR surveillance data are scarce in SSA. Here, we have used our comprehensive sentinel surveillance dataset to describe longitudinal trends in BSI and AMR over a 19-year period at a large teaching hospital in Blantyre, Malawi, with particular focus on prevalence of ESBL and FQR among Gram-negative pathogens and the emergence of MRSA.

## METHODS

### Study Setting

Blantyre is one of two principal cities in Malawi and during the study period, both its urban and peri-urban rural areas, had a rapidly expanding population, which was estimated at 1.3 million in 2016. There is a high burden of HIV, malaria and malnutrition, however during the study period antiretroviral therapy (ART) programmes, malaria control interventions and improvements in food security and community management of malnutrition were rolled out. HIV prevalence in Blantyre has declined from 22.3% in 2004 to 17·6% in 2016, whilst enrolment on the ART programme in Malawi has increased from 4,000 (2·3% of those in need of ART) in 2004 to over 530,000 (67·0% those in need of ART) in 2014. [20-23] Conjugate vaccine introductions against *Haemophilus influenza* type b (2002) and pneumococcus (2011) occured.[24-26] Over the period of BSI surveillance, there were also considerable reductions in mortality among children less than five years of age and among HIV-infected adults.[16,27,28]

QECH is one of the largest government hospitals in Malawi and is the only public hospital providing free medical care to Blantyre city, serving an urban population estimated at 920,000 in 2016. There are approximately 1,000 physical beds, though occupancy frequently exceeds capacity. The hospital admits approximately 10,000 adult (aged≥16 years) and 30,000 paediatric (aged< 16 years) medical patients/year. From 1998-2015, empirical management of sepsis was either with chloramphenicol and benzylpenicillin, or ceftriaxone, which was introduced in Malawi in 2004. Ceftriaxone was not widely available in the city or district of Blantyre outside of QECH, however it was extensively used as a first-line agent at QECH and was empirically given to 90·0% of febrile adult patients admitted to QECH in one study in 2009/10.[16]

### Diagnostic microbiology procedures

The Malawi-Liverpool-Wellcome Trust Clinical Research Programme (MLW) has provided routine, quality controlled, diagnostic blood culture (BC) service for febrile adult and paediatric medical patients admitted to QECH since 1998. A recommended seven to ten mls of blood were taken for culture under aseptic conditions from all adult patients admitted to the hospital with fever (axillary temperature > 37.5°C) or clinical suspicion of sepsis, severe sepsis or septic shock.[29] Clinicians were able to asses the following features of the systemic inflammatory

response syndrome and severe sepsis at a patient's bed-side in order to inform their decision to draw blood for culture; tachycardia (≥ 90 beats/minute), hypotension (systolic blood pressure < 90 mmHg), tachypnoea (respiratory rate > 20/minute) and delirium. The presence of fever and one or more of these features would lead the clinician to take a blood culture. A recommended three to ten mls of blood were also taken from children with the following criteria; non-focal febrile illness who tested negative for malaria, severely ill with suspected sepsis, or patients who failed initial malaria treatment and remained febrile.[8] In this extremely busy hospital afebrile patients were unlikely to have blood sampled for culture unless critically ill with suspected sepsis. The blood was inoculated into a single aerobic bottle (BacT/Alert, bioMérieux Marcy-L'Etoile, France).[6]

BC was undertaken using the automated BacT/ALERT system (bioMérieux, France) since 2000, prior to which manual culture was used and the method has previously been described.[30] Enterobacteriaceae and oxidase positive Gram-negative bacilli were identified by API (BioMérieux, France), *Staphylococci* by tube coagulase, ß-haemolytic *Streptococci* by Lancefield antigen testing and *Salmonellae* by serotyping according to the White-Kauffmann-Le Minor scheme by the following antisera; polyvalent O & H, O4, O9, Hd, Hg, Hi, Hm, and Vi antisera (Pro-Lab Diagnostics, UK). The identification of a sample of isolates identified as *Salmonella enterica* serovar Typhimurium was subsequently confirmed by whole genome sequencing and multilocus sequence typing. *Haemophilus influenza* were typed using type B antisera. Bacteria that form part of the normal skin or oral flora including: Diphtheroids, *Bacilli*., *Micrococci.*, coagulase negative *Staphylococci*, and alpha-haemolytic *Streptococci* (other than *S. pneumoniae*) were considered to be contaminants.[31]

Antimicrobial susceptibility tests were performed by the disc diffusion method following the British Society of Antimicrobial Chemotherapy (BSAC) methods and breakpoints (www.bsac.org.uk). Testing was in most cases limited to one plate containing six discs and the choice of agent has varied depending on the range of antimicrobials available to clinicians. The most current standard operating procedures are included in Supplementary Methods. Bacteria were defined as being resistant to Malawian first-line agents (hereafter, resistant to first-line or RFL) if they were resistant to the three first-line antimicrobial agents commonly used in Malawi, namely amoxicillin, cotrimoxazole and chloramphenicol for Gram-negative isolates, or penicillin, cotrimoxazole, and chloramphenicol for Gram-positive isolates. Isolates were

considered MDR if they were found to be resistant to three or more classes of antimicrobials to which reference strains are susceptible.[32] Gram negative isolates have been screened for ESBL-producing status using a cefpodoxime disc since 2007. Prior to this, ESBL was inferred based on resistance to ceftriaxone. ESBL was not confirmed. Methicillin resistance in *S. aureus* was inferred by cefoxitin resistance, which replaced oxacillin resistance testing in 2010.

### Statistics

We have reported prevalence of BC collection at MLW and causes of BSI in Blantyre using frequency distributions. Minimum annual incidence rates were expressed as incidence per 100,000 age-specific person years and estimated by dividing the number of BSI cases by mid-year population and multiplying by 100,000. We used the Cochran-Armitage test for trend and negative binomial regression to examine association between BSI incidence and time. Age stratified population estimates for urban Blantyre for the years 1998-2007 were obtained from the 1998 National Population Projections and for the years 2008-2016 from the 2008 National Population Projections by the National Statistical Office (NSO; http://www.nsomalawi.mw). Statistical analyses were performed using R Statistical Package version 3.1.2 for MacOS (R Core Team, www.r-project.org).

## ETHICS STATEMENT

MLW BC surveillance at QECH was approved by the College of Medicine Research Ethics Committee (COMREC) of the University of Malawi, approval number P.08/14/1614.

## ROLE OF FUNDING SOURCE

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit the paper for publication.

## RESULTS

In total, 194,539 blood cultures were collected from January 1998 to December 2016 from adult (79,095 [40·7%]) and paediatric (115,444 [59·3%]) patients presenting to QECH. The absolute number of BCs collected per year fluctuated during the surveillance, but we observed a declining

trend in ratio of BCs to population size, especially after 2005 (Figure 1A; p<0·0001). 29,183 (15·0%) BCs yielded pathogens (Table 1), a further 36,763 (18·9%) yielded contaminants (Figure 1A & Supplementary Figure 1) and there was no growth from 128,593 (66·1%) BCs (Figure 1A). Estimated incidence rate of BSI also declined substantially during the study period (Figure 1A; p<0·0001). The pathogen profiles in children and adults are shown in Supplementary Tables 1 & 2 respectively.

**Enterobacteriaceae**

Trends in *Salmonella* BSI revealing epidemics of NTS which peaked in 2002, and a more recent epidemic of Typhoid fever were previously described in detail up to 2014[6], but are included in Table 1 and Figure 1 to place other causes of BSI in context. Our analysis further shows that the *S.* Typhi epidemic, which had peaked in 2013, has been declining since 2013 (Figure 1B). A total of 2,560 *E. coli* isolates (8·8% BSI cases), 1,281 *Klebsiella spp.* isolates (4·4% BSI) and 1,130 isolates of other species from the Enterobacteriaceae family (3·9% BSI) were recorded between 1998-2016. The most common organisms in in the group of other Enterobacteriaceae species were *Enterobacter spp*., 517, *Serratia spp*., 211, *Citrobacter spp*. 185, *Proteus spp*., 93, and *Shigella spp*., 40 (Table 1). Incidence of BSI attributable to these pathogens significantly declined over time (Figure 1B & C; p<0·0001).

**Other Gram-negative pathogens**

Other Gram-negative causes of BSI included *Acinetobacter spp.* (543), *Haemophilus spp.* (434), *Pseudomonas spp.* (442), *Neisseria spp.* (210), other Anaerobes (30) and *Vibrio spp.* (12). These together were responsible for 1,671 (5·7%) positive blood cultures (Figure 1E).

***S. pneumoniae***

Trends in *S. pneumoniae* BSI were previously described for the period 2000-2009,[8] whereas this dataset spans 1998-2016. In total, there were 4,258 *S. pneumoniae* isolates (14·6% BSI) during the period 1998-2016. We observed a significant downward trend in the annual incidence of BSI due to *S. pneumoniae* (Figure 1B; p<0·0001).

***S. aureus***

*S. aureus* was the second most common Gram-positive cause of BSI with 1,923 (6·6% BSI) isolates during the study period. The isolation frequency of *S. aureus* fluctuated throughout the study period (Figure 1D), but the incidence of *S. aureus* BSI declined significantly ($p<0.0001$).

**Other Gram-positive pathogens**

There were a total of 1,193 (4·5% BSI) β-haemolytic *Streptococcus spp.,* including 397 (1·4% BSI) isolates of Lancefield Group A and 477 (1·6% BSI) isolates of Group B *Streptococcus spp.* and 320 *Enterococcus spp* (1·0% BSI) of which 220 (0·8% BSI) isolates were *E. faecalis,* 76 (0·3% BSI) isolates were *E. faecium* and 42 (0·1% BSI) isolates were other *Enterococcus spp*. Whereas the incidence of β-haemolytic *Streptococci* declined ($p=0·0005$) during the surveillance, there was no significant change in *Enterococci* BSI ($p=0·4900$).

**Yeasts**

Our longitudinal bacteraemia surveillance also led to the isolation of 1003 isolates (3.4% BSI) of yeast (Table 1), including 963 *Cryptococcus neoformans* and 40 *Candida* species. After consistently increasing between 1998 and 2006, the incidence of yeast BSI has been in decline since 2006 (Figure 1D).

**Age Distribution**

Of the 29,183 episodes of culture-confirmed BSI, age was known in 23,219 (79·6%) cases. 13,002 (56·0%) cases with known age were children (<16 years) and 8,621 (44·0%) were adults (≥16 years old). 10,059 (77·4%) children were aged below five years. Most bacterial species associated with BSI in Blantyre displayed a bimodal age distribution, affecting mostly children under 5 years of age and adults aged between 20-45 years (Supplementary Figure 2). The only exception was *S*. Typhi, which was most common in children under 10 years of age.[6] Cryptococcal BSI was most common in adults aged between 20 and 45 years, but uncommon in children (Supplementary Figure 2).

When the aggregate data were adjusted by age distribution in the population to produce minimum incidence estimates stratified by age, a subtly different picture emerged. It was noted that the minimum incidence rates for *E. coli* BSI were greatest in older age groups ≥ 70 years (up to 54·3/100,000/year) followed by children aged below five years (37·5/100,000/year); and

for *Klebsiella* BSI, incidence rates were highest in children below five years (29·2/100,000/year) followed by the elderly aged 75-80 years (24·6/100,000/year). For *S.* Typhi, incidence rates were greatest in children between 5-10 years and for all other bacterial pathogens they were greatest in those below 5 years of age (Figure 2).

**Trends in Antimicrobial resistance**

27,249/28,180 (96·7%) confirmed bacterial BSI isolates were tested for susceptibility to at least one antimicrobial agent and 25,752 (91·4%) BSI isolates were tested for susceptibility to at least three antimicrobial agents. 13,343/25,572 (52·2%) isolates tested for susceptibility to at least three agents were RFL, 10,316/25,572 (40·3%) were resistant to one or two first-line agents, and 2,093 isolates (8·2%) were susceptible to all three first-line agents (Figure 3). Overall, proportions of RFL isolates followed an upward trend during the surveillance period (Supplementary Figure 3A; p<0.0001). RFL was markedly more common among Gram-negative isolates (12,902/18,887 [68·3%]) than Gram-positive isolates (441/6,685 [6·6%]). 6,129/6,685 (91·7%) of all Gram-positive isolates were susceptible to either penicillin or chloramphenicol.

**Partial return of chloramphenicol susceptibility in *E. coli* and *Klebsiella spp.***

In contrast to the overall trends in RFL isolates, the proportion of *E. coli* that were RFL substantially declined during the study period (p<0·0001), primarily due to a decline in chloramphenicol resistance (Figure 3A; p<0·0001). Chloramphenicol resistance also declined in in *Klebsiella spp.* (p<0·0001), but increased in *Salmonellae* (Supplementary Figure 3B) , whilst no significant trend was detected in other members of the Enterobacteriaceae family ( Figure 3C; p=0·2203).

**Emergence of ESBL producing, fluoroquinolone and aminoglycoside resistant isolates**

ESBL production was first detected in *E. coli* in 2004 and in *Klebsiella spp*. and other Enterobacteriaceae in 2003. Both frequency and incidence of ESBL- producing isolates have since increased markedly in all non-*Salmonellae* Enterobacteriaceae (Figure 4; Supplementary Figure 4). In addition to the Enterobacteriaceae, a majority (168/274 [61·3%]) of *Acinetobacter spp.* isolates were ESBL producers and there was also an increasing trend of ESBL *Acinetobacter spp*. isolates (Supplementary Figure 5). Ceftazidime was not widely available, therefore *Pseudomonas* isolates were not routinely tested, however 7/9 *Pseudomonas* isolates

tested were resistant to ceftazidime.

Ciprofloxacin resistance was first detected in Blantyre in *Acinetobacter* isolates (1/43 [2·3%]) in 2001 and in *E. coli* (4/22 [2·5%)] and *Klebsiella* isolates in 2003. As with ESBL resistance, we observed an increasing trend in both the proportion and rate of non-*Salmonellae* Enterobacteriaceae with resistance to fluoroquinolones (Figure 4). 105/393 (26·7%) and 55/344 (12·8%) of all *Acinetobacter* and *Pseudomonas* isolates were ciprofloxacin resistant.

During the surveillance, we also detected 462/2536 (18·2%) *E. coli*, 565/1265 (51·9%) *Klebsiella spp.*, and 320/1076 (29·7%) of the other Enterobacteriaceae resistant to gentamicin with substantial increases in proportions of resistant isolates observed over time (Figure 4). In other Gram-negative pathogens, 210/524 (40·1%) *Acinetobacter* and 140/417(33·6%) *Pseudomonas* isolates were also resistant to gentamicin.

**Antimicrobial resistance in Gram-positive pathogens**

***S. pneumoniae***

Only 37/3,049 (0·9%) *S. pneumoniae* isolates were resistant to all Malawian first-line agents (Figure 3D). Resistance to cotrimoxazole was highly prevalent (3,780/4,087 isolates [92·5%]). In contrast, only 610/4,043 (15·1%) isolates were resistant or intermediate resistant to penicillin (falling to 551 [13·6%] when intermediate resistance was excluded). The overall trend in penicillin resistant isolates was not significant (p=0.2300) but a marked rise was observed following the introduction of the pneumococcal conjugate vaccine (PCV13) in 2011 (Figure 3D; p<0·0001). 1,074/4,111 (26·1%) isolates were resistant to chloramphenicol. Of isolates tested for susceptibility to both chloramphenicol and penicillin, 3,971/4,013 (99·0%) were susceptible to at least one of the two antimicrobial agents. We also identified 2,200/4,080 (53·9%) *S. pneumoniae* isolates that were resistant to tetracycline and 92/41,07 (2·2%) *S. pneumoniae* isolates that were resistant to the macrolide erythromycin.

**Emergence of MRSA**

In total, 1,151/1,457 (79·0%) *S. aureus* isolates were resistant to penicillin whilst 828/1895 (43·7%) were resistant to cotrimoxazole and 452/1898 (23·8%) to chloramphenicol. 107/1,118

(9·6%) *S. aureus* isolates were MRSA, which was first detected in 1998, but was not regularly isolated until 2005 (Figure 3E). Only 20/1,681 *S. aureus* isolates were tested for susceptibility to ciprofloxacin and none was resistant. 206/1,790 (11·5%) isolates tested were resistant to gentamicin.

**Other Streptococcus and *Enterococcus spp.***

855/1,213 (70·5%) of the β-haemolytic *Streptococci* isolates and 229/325 (70·5%) *Enterococci* isolates were resistant to cotrimoxazole, whereas 176/1149 (15·3%) and 108/176 (61·4%) *Streptococci* and *Enterococci spp.* were resistant to penicillin and ampicillin respectively 208/1143 (17·1%) *Streptococci* and 198/326 (60·7%) *Enterococci* were resistant to chloramphenicol. Amongst Group A *Streptococci*, 14/382 (3·7%) were penicillin resistant, however these isolates were not speciated and we are therefore unable to specifically identify the *Streptococcus pyogenes* isolates within this group.

**Multi-drug resistant and untreatable pathogens**

The majority of *Salmonellae*, *E.coli, Klebsiella,* and indeed all other Enterobacteriaceae were MDR, as were a substantial proportion of *S. pneumoniae,* other *Streptococci* and *Enterococci* Table 2. Trend in MDR isolates was increasing in *Klebsiella spp.* ($p<0·0001$) and in other *Streptococcus spp.* and *Enterococcus spp.* ($p<0.0001$). We however, detected a declining MDR trend ( $p<0.0001$) in *E. coli* isolates (Table 2).

381 Gram-negative isolates were resistant to all agents tested against, including amoxicillin, cotrimoxazole, chloramphenicol, gentamicin, ciprofloxacin or ceftriaxone, rendering them locally untreatable. These included 121/381 (31·8%) *Klebsiella spp.,* 68/381 (17·8%) *E. coli*, 119/381 (26·0%) assorted other Enterobacteriaceae and 119/381 (31·2%) *Acinetobacter* (Figure 4D). The number of isolates expressing resistance to all the six agents was 81/1,106 (7·3% BSI) in 2016 from 2/2,372 (<0.1%) in 2003 (Figure 4D).

**DISCUSSION**

Long term sentinel surveillance in Malawi has revealed a marked decline in the incidence of BSI caused by all pathogens between 1998 and 2016. Concurrent with the declining incidence however, has been an increase in the prevalence of antimicrobial resistance - including to reserve

antimicrobials. These changes in the incidence of BSI and the emergence of AMR occurred against a background of improvements in food security, malaria control interventions and highly successful roll-out of ART. Following the emergence of widespread resistance to commonly available first-line antimicrobial agents, cephalosporins and fluoroquinolones have become the agents of choice for treatment of severe bacterial infections in SSA including Malawi.[33] The emergence and spread of ESBL resistance and FQR is therefore a major worry in this setting. The rates of ESBL resistance and FQR of up to 30·1% and 31·1% in *E. coli* and 90·5% and 70·2% in *Klebsiella spp.* reported here are very high, whether placed in an African or a global context.[34-36] Moreover, in the other settings ESBL and FQR have been reported to be more common in hospital acquired than community acquired infections, whereas this study reflects community acquired bacteraemia.[37,38]

Minimum incidence estimates suggest that *E. coli* and *Klebsiella* BSI are particularly prominent in elderly patients, consistent with global data.[39] The Malawi NSO estimates that life expectancy in Blantyre will increase from ~55 years in 2007 to ~70 years by 2030 (http://www.nsomalawi.mw). Increased life expectancy may increase the pool of persons at risk of *E. coli* and *Klebsiella* BSI, with the attendant risk of drug resistance increasing the prominence of these pathogens with time.

Resistance to first line antimicrobials has fluctuated. In some Enterobacteriaceae such as *E. coli* and *Klebsiella spp*., RFL rates have begun to decline, primarily due to less chloramphenicol resistance. However, the molecular determinants of this observation in *E. coli* and *Klebsiella spp.* have not been ascertained. This partial re-emergence of chloramphenicol susceptibility is not sufficiently great to permit its reintroduction as an agent for the empirical management of sepsis in Blantyre, due to widespread resistance amongst *Salmonellae*, the dominant gram negative pathogens.

Chloramphenicol and penicillin have been commonly used in combination for the empirical management of sepsis in Malawi for many years.[8] It is interesting to note that almost all (99·0%) of the *S. pneumoniae* isolates are still susceptible to this combination despite its wide usage. However, it is equally important to note that penicillin resistance has started to increase since the introduction of PCV13 in 2011. PCV13 introduction has been associated with a general decline

in penicillin resistant *S. pneumoniae* in South Africa.[40] However, increasing prevalence of penicillin resistant *S. pneumoniae* serotype 19F and non-vaccine serotypes such as 19A and 15A have been reported following the introduction of the PCV7 and PCV13 outside SSA.[41,42] The increase in the proportion *S. pneumoniae* that are penicillin resistant at a time when *S. pneumoniae* BSI is in decline raises the possibility that there has been a change in serotype distribution following vaccine introduction as has been the case in the other settings.[41,42]

In this study, we also describe the emergence of MRSA, yet it currently remains an infrequent cause of BSI in Blantyre. Prevalence of MRSA amongst the *S. aureus* isolates is similar to proportions from countries such as Mozambique and Zimbabwe, but much lower to those reported in South Africa.[43-45] This may be a reflection of the fact that our cultures were taken from community admissions to medical wards; we have yet to study nosocomial infection in depth in our setting. It might also reflect that unlike in South Africa, medical devices placing patients at risk of MRSA BSI such as central venous catheters are uncommonly used in low income countries such as Malawi. The sustained presence of MRSA as a low-level cause of BSI in Blantyre is therefore of considerable concern as its relative importance as a BSI pathogen may greatly change if surveillance expands to cover surgical patients or nosocomial infections or if medical practice changes, for example a renal dialysis unit is under development at QECH.

**Limitations**

This study describes a comprehensive and extensive dataset from a sustained period of bacteraemia surveillance, however it has a number of limitations. The median length of stay for adult internal medicine inpatients is five days and is shorter for children.[46] Typically patients at QECH undergo BC on admission but it was uncommon for patients to have follow-up BC and it is therefore unlikely that our surveillance has captured much nosocomial infection. It is possible that community acquired sepsis was missed if persons died at home or were not referred to hospital, hence we consider rates reported to be minimum estimates.

Antimicrobial susceptibility profile was described for 96·7% of the isolates and reflect BSAC guidelines at time of testing. ESBL screening was not introduced until 2003, and as such ESBL-producing pathogens may have been circulating, but undetected before then. ESBL screening by cefpodoxime disc testing was not introduced until 2007, consequently some isolates may have

been falsely classified as ESBL producing prior to 2007. In the case of *S. aureus*, cefoxitin screening for MRSA replaced methicillin in 2010, although the small increase in sensitivity gained will have made minimal difference to the reported findings.

## CONCLUSIONS

The overall declines in bacterial BSI have been accompanied by a rise in antimicrobial resistance in all bacterial BSI pathogens at QECH, especially in Gram-negative organisms, and the emergence of methicillin resistance in *S. aureus.* Ceftriaxone and ciprofloxacin have been essential for the management of bacterial BSI in a context where human immunosuppression and bacterial multidrug resistance are highly prevalent. The emergence of ESBL, fluoroquinolone and gentamicin resistance and MRSA highlight the growing challenge of BSIs that are effectively impossible to treat in this resource-limited setting.

## AUTHOR CONTRIBUTIONS

P.M., C.L.M., R.S.H., D.B.E. and N.A.F. conceived and designed the study. C.L.M., R.S.H., D.B.E. and N.A.F. supervised the study. B.D., C.M., M.A.G., N.F., D.B.E., R.S.H. and N.A.F. collected and provided data; P.M. analysed the data, prepared tables and figures. P.M. and N.A.F. interpreted the results and drafted the manuscript. P.M., J.E.C., J.M. N.K., C.M., B.D., N.B., M.A.G., N.F., R.S.H., C.L.M., D.B.E., N.A.F. contributed to the discussions and commented on the manuscript. All the authors have read and approved the final manuscript.

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## DECLARATION OF INTEREST

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## FIGURE LEGENDS

**Figure 1:** Trends in BSI 1998-2016. 1A: Annual frequency of blood culture sampling, and pathogen and contaminant isolation, plus estimated minimum incidence rates of BSI. 1B-D Estimated minimum incidence of pathogens isolated at high frequency (≥300/year -1B) and intermediate frequency (50-299/year - 1C & 1D). Pathogens isolated at low frequency (<50 year) are depicted in 1E.

**Figure 2**: Estimated minimum incidence rates of BSI stratified by age for (2A) *S*. Typhimurium (2B) *S*. Enteritidis (2C) *S*. Typhi, (2D) *E. coli,* (2E) *Klebsiella spp*., (2F) other Enterobacteriaceae, (2G) *S. pneumoniae,* (2H) *S. aureus* (2I) Yeast.

**Figure 3**: Trends in proportions of isolates resistant to Malawian first line antimicrobials for (3A) *E. coli,* (3B) *Klebsiella spp.,* and (3C) other Enterobacteriaceae, (3D) *S. pneumoniae,* (3E)*, S. aureus* and (3F) other *Streptococcus/Enterococcus spp.* First line antimicrobials include chloramphenicol and cotrimoxazole, plus ampicillin for gram negative pathogens and penicillin for gram positive pathogens.

**Figure 4:** Trends in resistance to second-line antimicrobial agents (ciprofloxacin, ceftriaxone and gentamicin) in (A) *E. coli*, (B) *Klebsiella spp*. and (C) other Enterobacteriaceae and (D) trend in number of isolates resistant to all six commonly used antimicrobial agents in Malawi (*ampicillin, chloramphenicol, cotrimoxazole, ceftriaxone, ciprofloxacin and gentamicin).

## TABLES

**Table 1: Prevalence of significant pathogens in four time intervals: 1998-2001, 2002-2005 , 2006-2009, 2010-2013 and 2014-2016.**

| Organism | No. of isolates (%) | | | | | |
|---|---|---|---|---|---|---|
| | **1998-2001** | **2002-2005** | **2006-2009** | **2010-2013** | **2014-2016** | **Total** |
| ***Acinetobacter spp.*** | 200 (3·0) | 126 (1·0) | 115 (2·0) | 64 (1·5) | 40 (1·0) | **545 (1·9)** |
| **Anaerobes** | 7 (0·0) | 8 (0·0) | 7 (0·0) | 6 (0·1) | 2 (0·1) | **30 (0·1)** |
| ***Citrobacter spp.*** | 69 (1·0) | 75 (0·8) | 20 (0·4) | 9(0·2) | 12 (0·3) | **185 (0·6)** |
| ***E. coli*** | 592 (9·0) | 661 (7·0) | 552 (10·0) | 398 (9·3) | 357(9·3) | **2560 (8·8)** |
| ***E. faecalis*** | 48 (0·7) | 57 (0·6) | 61 (1·1) | 27 (0·6) | 27(0·7) | **220 (0·8)** |
| ***Edwardsiella spp.*** | - | 2 (0·0) | - | - | - | **2 (0·0)** |
| ***Enterobacter*** | 93 (1·3) | 173 (2·0) | 88 (1·6) | 74 (1·7) | 89(2·3) | **517 (1·8)** |
| ***Enterococcus spp.*** | 14 (0·2) | 2 (0·0) | 2 (0·0) | 64 (1·5) | 69 (1·8) | **151 (0·5)** |
| ***Escherichia spp.*** | - | 3 (0·0) | 3 (0·1) | - | - | **6 (0·0)** |
| ***Flavobacteria spp.*** | 2 (0·0) | 2 (0·0) | 2 (0·0) | - | - | **6 (0·0)** |
| ***H. influenza type b*** | 112 (1·6) | 92 (1·0) | 26 (0·5) | 30 (0·7) | 15 (0·4) | **275 (0·9)** |
| ***Haemophilus spp.*** | 41 (0·6) | 53 (0·6) | 36 (0·7) | 21 (0·5) | 8 (0·2) | **434 (0·5)** |
| ***Hafnia spp.*** | 3 (0·0) | 2 (0·0) | 1 (0·0) | - | | **6 (0·0)** |
| ***Klebsiella spp.*** | 449 (7·0) | 248 (3·0) | 211 (4·0) | 190 (4·4) | 183 (4·8) | **1281 (4·4)** |
| ***Kluyvera spp.*** | 2 (0·0) | 1 (0·0) | 2 (0·0) | 2 (0·0) | - | **7 (0·0)** |
| ***M. morganii*** | - | 3 (0·0) | 4 (0·1) | 11 (0·3) | 1 (0·0) | **19 (0·1)** |
| ***Mycobacterium*** | 60 (1·0) | - | - | - | | **60 (0·2)** |
| ***Neisseria*** | 78 (1·0) | 27 (0·0) | 42 (1·0) | 34 (0·8) | 39(1·0) | **220 (0·8)** |
| **Other Gram Negative cocci** | 15 (0·0) | 11 (0·0) | 11 (0·2) | 1 (0·0) | - | **38 (0·1)** |
| **Other Gram-negative rods** | 18 (0·0) | 45 (1·0) | 41 (1·0) | 55 (1·3) | 7 (0·2) | **166 (0·6)** |
| **NTS** | 2,685 (38·9) | 4,432 (50·1) | 2,141 (40·2) | 782 (18·3) | 433 (11·3) | **10473 (35·9)** |
| ***Streptococcus spp.*** | 115 (1·7) | 113 (1·3) | 55 (1·0) | 46 (1·1) | 57 (1·5) | **386 (1·3)** |
| ***Pantoea spp.*** | - | - | - | 11(0·3) | 9 (0·2) | **20 (0·1)** |
| ***Proteus spp.*** | 47 (0·7) | 9 (0·1) | 12 (0·2) | 7 (0·2) | 18 (0·5) | **93 (0·3)** |
| ***Pseudomonas*** | 98 (1·0) | 102 (1·0) | 41 (1·0) | 126 (2·9) | 75 (2·0) | **442 (1·5)** |
| ***Raoultella spp.*** | - | - | - | 3 (0·1) | 8 (0·2) | **11 (0·0)** |
| ***S. agalactiae*** | 173 (2·5) | 155 (1·8) | 40 (0·8) | 70 (1·3) | 17 (0·4) | **455 (1·6)** |
| ***S. aureus*** | 505 (7·0) | 480 (5·0) | 258 (5·0) | 344 (8·0) | 338 (8·8) | **1925 (6·6)** |
| ***S. pneumoniae*** | 1,139 (17·0) | 1,476 (17·0) | 1,072 (20·0) | 448 (10·5) | 123 (3·2) | **4258 (14·6)** |
| **Group A *Streptococcus*** | 117 (1·7) | 102 (1·2) | 69 (1·3) | 59(1·4) | 50 (1·3) | **397 (1·4)** |
| ***S.* Typhi** | 67 (1·0) | 49 (1·0) | 70 (1·0) | 1168 (27·3) | 1643 (43·0) | **2997(10·3)** |
| ***Serratia spp.*** | 92 (1·3) | 66 (0·7) | 15 (0·3) | 21 (0·5) | 17 (0·4) | **211(0·7)** |
| ***Shigella spp.*** | 1 (0·0) | 9 (0·1) | 18 (0·3) | 6 (0·1) | 6 (0·2) | **40 (0·1)** |
| ***Vibrio spp.*** | 5 (0·0) | 2 (0·0) | 2 (0·0) | 3 (0·1) | - | **12 (0·0)** |
| ***Yersinia*** | 2 (0·0) | 2 (0·0) | 1 (0·0) | 2 (0·0) | - | **7 (0·0)** |
| ***Candida spp.*** | 4 (0·0) | 6 (0·1) | 4 (0·1) | 13 (0·3) | 13 (0·3) | **40 (0·1)** |
| ***Cryptococcus spp.*** | 50 (1·0) | 249 (2·8) | 300 (5·6) | 195 (4·6) | 169 (4·4) | **963 (3·3)** |
| **Total** | **6,903** | **8,843** | **5,322** | **4290** | **3825** | **29183** |

**Table 2: Trends in frequency of MDR bacterial BSI pathogens in Blantyre between 1998 and 2016. Isolates are considered MDR when found to be resistant to at least three antimicrobial classes.**

| Year | *E. coli* | *Klebsiella spp.* | Enterobacteriaceae | *S. pneumoniae* | *Enterococcus spp.* | *Streptococcus spp.* |
|---|---|---|---|---|---|---|
| **1998** | 111/185(60) | 66/146 (45·2) | 22/30 (73·3) | 85/335 (25·4) | 18/26 (69·2) | 18/90 (20·0) |
| **1999** | 118/150(78·7) | 31/113 (27·4) | 12/26 (46·2) | 98/313 (31·3) | 8/13 (61·5) | 15/93 (16·1) |
| **2000** | 83/116 (71·6) | 38/101 (37·6) | 60/90 (66·7) | 80/238 (33·6) | 3/8 (37·5) | 21/143 (14·7) |
| **2001** | 96/124 (77·4) | 17/73 (23·3) | 87/131 (66·4) | 41/154 (26·6) | 10/15 (66·7) | 13/70 (18·6) |
| **2002** | 113/147 (76·9) | 20/81 (24·7) | 64/80 (80·0) | 75/231 (32·5) | 2/7 (28·6) | 8/72 (11·1) |
| **2003** | 110/152 (72·4) | 24/52 (46·2) | 67/108 (62·0) | 116/316 (36·7) | 2/3 (66·7) | 13/74 (17·6) |
| **2004** | 92/133 (69·2) | 21/46 (45·7) | 39/62 (62·9) | 83/265 (31·3) | 5/7 (71·4) | 22/79 (27·8) |
| **2005** | 121/181 (66·9) | 22/46 (47·8) | 24/55 (43·6) | 158/494 (32·0) | 32/41 (78·0) | 28/114 (24·6) |
| **2006** | 84/186 (45·2) | 18/52 (34·6) | 45/76 (59·2) | 110/413 (26·6) | 6/13 (46·2) | 15/70 (21·4) |
| **2007** | 81/136 (59·6) | 27/57 (47·4) | 15/31 (48·4) | 105/320 (32·8) | 9/14 (64·3) | 10/46 (21·7) |
| **2008** | 81/116 (69·8) | 36/55 (65·5) | 10/27 (37·0) | 54/160 (33·8) | 12/14 (85·7) | 6/29 (20·7) |
| **2009** | 60/106 (56·6) | 29/40 (72·5) | 12/22 (54·5) | 47/151 (31·1) | 12/17 (70·6) | 4/16 (25·0) |
| **2010** | 64/96 (66·7) | 34/45 (75·6) | 25/34 (73·5) | 34/119 (28·6) | 10/13 (76·9) | 13/28 (46·4) |
| **2011** | 76/109 (69·7) | 35/48 (72·9) | 10/15 (66·7) | 71/177 (40·1) | 6/7 (85·7) | 28/71 (39·4) |
| **2012** | 42/86 (48·8) | 32/38 (84·2) | 17/27 (63·0) | 30/106 (28·3) | 15/16 (93·8) | 7/41 (17·1) |
| **2013** | 74/100 (74·0) | 33/55 (60·0) | 42/61 (68·9) | 12/41 (29·3) | 15/17 (88·2) | 25/58 (43·1) |
| **2014** | 63/105 (60·0) | 41/51 (80·4) | 40/57 (70·2) | 12/31 (38·7) | 15/18 (83·3) | 16/53 (30·2) |
| **2015** | 65/118 ( 55·1) | 39/48 (81·3) | 10/33 (30·3) | 25/50 (50·0) | 29/34 (85·3) | 19/46 (40·3) |
| **2016** | 92/133 (69·2) | 77/84 (91·7) | 51/65 (78·5) | 9/42 (21·4) | 37/48 (77·1) | 8/31 (25·8) |
| **Overall** | 1626/2479 (65·6) | 640/1231 (52·0) | 652/932 (63·3) | 1245/3956 (31·5) | 246/331 (74·3) | 289/1224 (23·6) |
| **p-value** | <0·0001* | <0·0001** | 0·747 | 0·148 | <0.0001** | <0·0001** |

*= decreasing trend; **=increasing trend.

## RESEARCH IN CONTEXT PANEL

### Evidence before this study

Long-term surveillance data describing bloodstream infection (BSI) and antimicrobial resistance (AMR) in sub-Saharan Africa (SSA) are scarce. A systematic review and meta-analysis of community-acquired BSI in Africa by Reddy et al identified only 22 studies over a period of more than 20 years (up to June 2009) and the majority (13) focused on children. Only one study from Egypt and none from SSA reported long-term (1999-2003) surveillance data for both children and adults, and only 13 studies reported antimicrobial susceptibility data.

A review of more recent literature available on PubMed (search strategy: ("Africa") AND ("community acquired") AND ("bacteraemia" OR "sepsis")) for the period July 2009-June 2016 revealed two large bacteraemia datasets; one from South Africa (Dramowski et al) containing 17,001 blood culture results from a 6 year period and the other from Mozambique (Mandomando et al) containing a further 19,896 blood culture results from a 5 year period. These studies reported trends in community acquired BSI and AMR in all pathogens, but only for paediatric patients. We found no studies detailing longitudinal passive BSI surveillance from both adults and children.

### Added value of this study

Like many sub-Saharan African countries, Malawi is under considerable pressure from poverty, undernutrition, urbanization and malaria. In addition, in the context of a high HIV seroprevalence, ART rollout has been highly successful. Blantyre is a major African city, grappling with the same issues of rapid expansion in population size with insufficient access to water and sanitation as elsewhere on the continent. Our data are therefore representative of urban Africa.

We present the largest bacteraemia and AMR surveillance dataset yet collected from SSA and describe trends in BSI in both adults and children presenting to a major urban teaching hospital in Malawi over a 19-year period. Our study reveals a marked decline in the incidence of BSI caused by all pathogens except *S.* Typhi. This has occurred concurrently with a number of major public health interventions, including the extensive roll out of both antiretroviral therapy and malaria control interventions, improvements in food security and the community management of malnutrition, and the introduction of *Haemophilus* influenza type b and pneumococcal conjugate

vaccines. This good news is tempered by the emergence and rapid expansion of drug resistant pathogens, including cephalosporin and fluoroquinolone resistant Enterobacteriaceae, penicillin resistant *S. pneumoniae* and methicillin resistant *S. aureus*. These surveillance data are critical to improving the understanding of the burden of severe, drug resistant bacterial infection in SSA.

**Implications**

Although there has been a marked fall in community-acquired bacterial BSI in Malawi, an increasing number of pathogens are becoming effectively untreatable due to their resistance to locally available antimicrobial agents.

## SUPPLEMENTARY APPENDIX

**Supplementary Table 1**: Distribution of isolates from children with BSI by pathogen and year of isolation

**Supplementary Table 2:** Distribution of isolates from adults with BSI by pathogen and year of isolation

**Supplementary Table 3:** Distribution of isolates by pathogen resistance profile and year of isolation.

**Supplementary Figure 1:** Distribution of contaminants as proportions of all blood cultures (BC)

**Supplementary Figure 2:** Age distributions of patients presenting to QECH between 1998 and 2016 with confirmed BSI caused by (A) *S.* Typhimurium, (B) *S.* Enteritidis, (C) *S.* Typhi, (D) *E. coli*, (E) *Klebsiella spp*., (F) other Enterobacteriaceae, (G) *S. pneumoniae*, (H) *S. aureus* and (I) yeast.

**Supplementary Figure 3**: Trends in resistance to first-line agents amongst in (A) all bacterial pathogens and (B) all *Salmonellae* isolates**.** As with Figure 3**,** first line antimicrobials include chloramphenicol and cotrimoxazole, plus ampicillin for gram negative pathogens and penicillin for gram positive pathogens.

**Supplementary Figure 4:** Trends in annual incidence (per 100,000) of Enterobacteriaceae resistant to ceftriaxone (CRO-R) or ciprofloxacin (CIP-R). A. Annual incidence of CRO-R *E. coli*; B. Annual incidence of CIP-R *E. coli*; C. Annual incidence of CRO-R *Klebsiella spp*; Annual incidence of CIP-R *Klebsiella spp.* E. Annual incidence of Other CRO-R Enterobacteriaceae; F. Annual incidence of other CIP-R Enterobacteriaceae. In For each panel, points represent observed annual incidence rates while the black line graph represent annual incidence rates predicted by a linear regression model with the general form $\boldsymbol{y = \beta_0 + \beta_1 x}$, where $\boldsymbol{y}$ is the incidence rate, $\boldsymbol{x}$ is the year of isolation and $\boldsymbol{\beta_0}$ and $\boldsymbol{\beta_1}$ are constant coefficients.

**Supplementary Figure 5:** Trends in proportions of *Acinetobacter spp.* isolates resistant to ceftriaxone, ciprofloxacin and gentamicin during 1998-2016.

**PANELS**

**Panel 1: Research in context**

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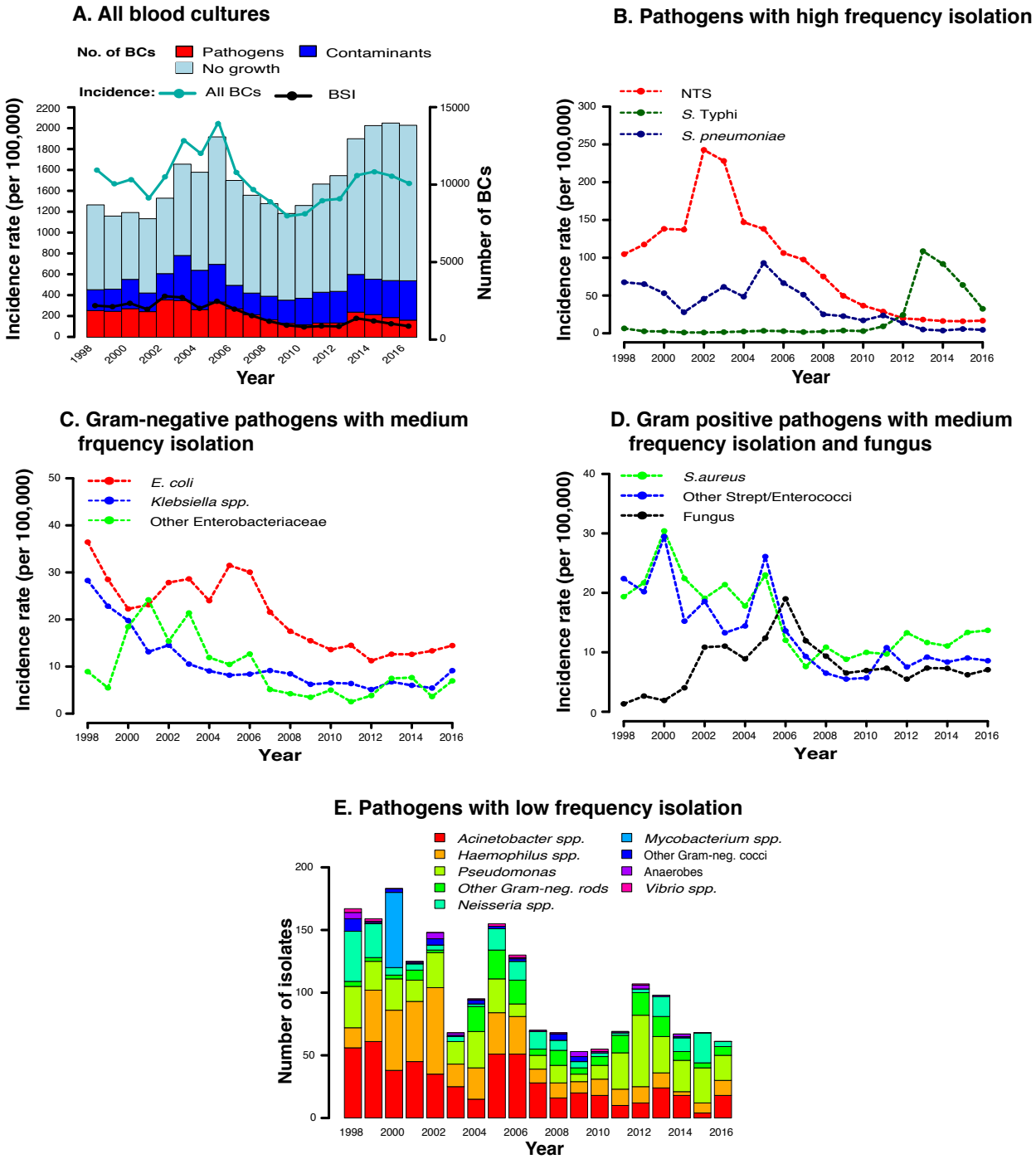


**Figure 1: Distributions of blood culture samples**. Year by year distribution of all blood culture samples by category is depicted in Figure 1A while distributions of blood cultures which grew into significant pathogens are shown in Figures 1B-E, (Pathogens were classified into four groups of high frequency isolation, (≥ 300 isolates in any year – Figure 1B), Gram-negative medium frequency isolation pathogens and Gram-positive medium isolation pathogens and yeast (Figure 1C and Figure 1D, with 50-299 isolates/year and low frequency isolation pathogens <50 isolates/year).

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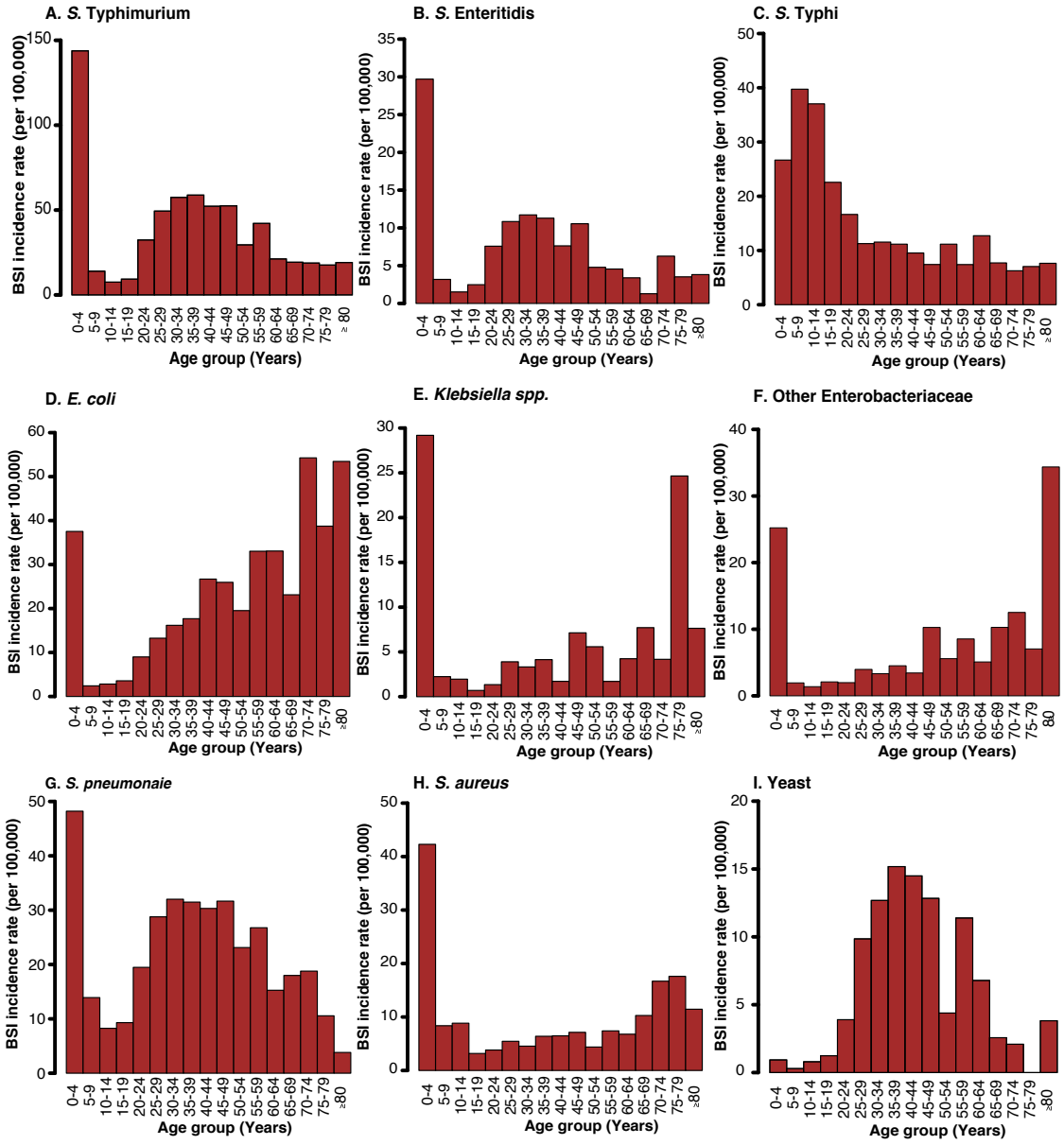


**Figure 2: Estimated minimum incidence rates of BSI stratified by age for** (A) *S.* Typhimurium, (B) *S.* Enteritidis (C) *S.* Typhi, (D) *E. coli,* (E) *Klebsiella spp*., (F) other Enterobacteriaceae, (G) *S. pneumoniae*, (H) *S. aureus* (I) Yeast.

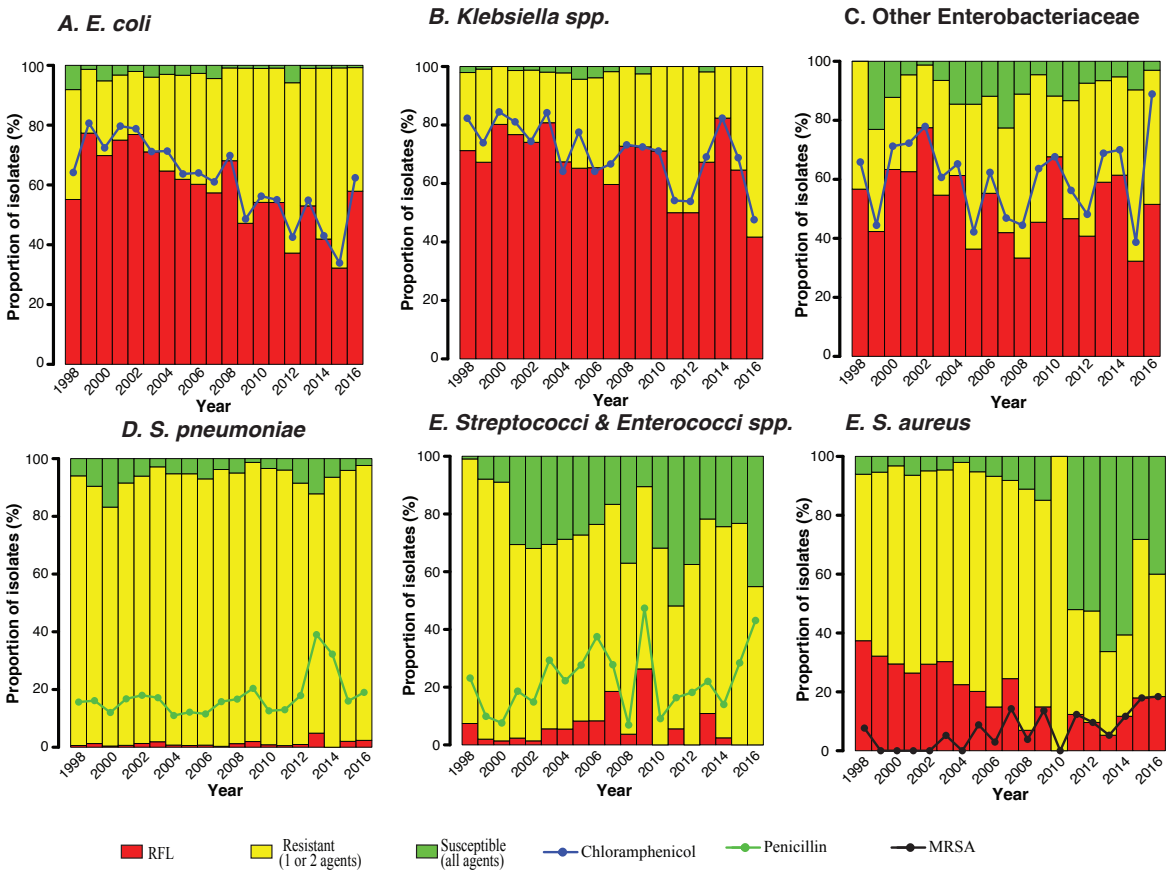


**Figure 3: Trends in proportions of RFL isolates (resistant to chloramphenicol, cotrimoxazole and ampicillin) for** (A) E. coli (B) Klebsiella spp. and (C) other Enterobacteriaceae and trends in proportions of RFL isolates (resistant to chloramphenicol, cotrimoxazole and penicillin) for (D) S. pneumonia (E), S. aureus and (F) other Streptococcus/Enterococcus spp.

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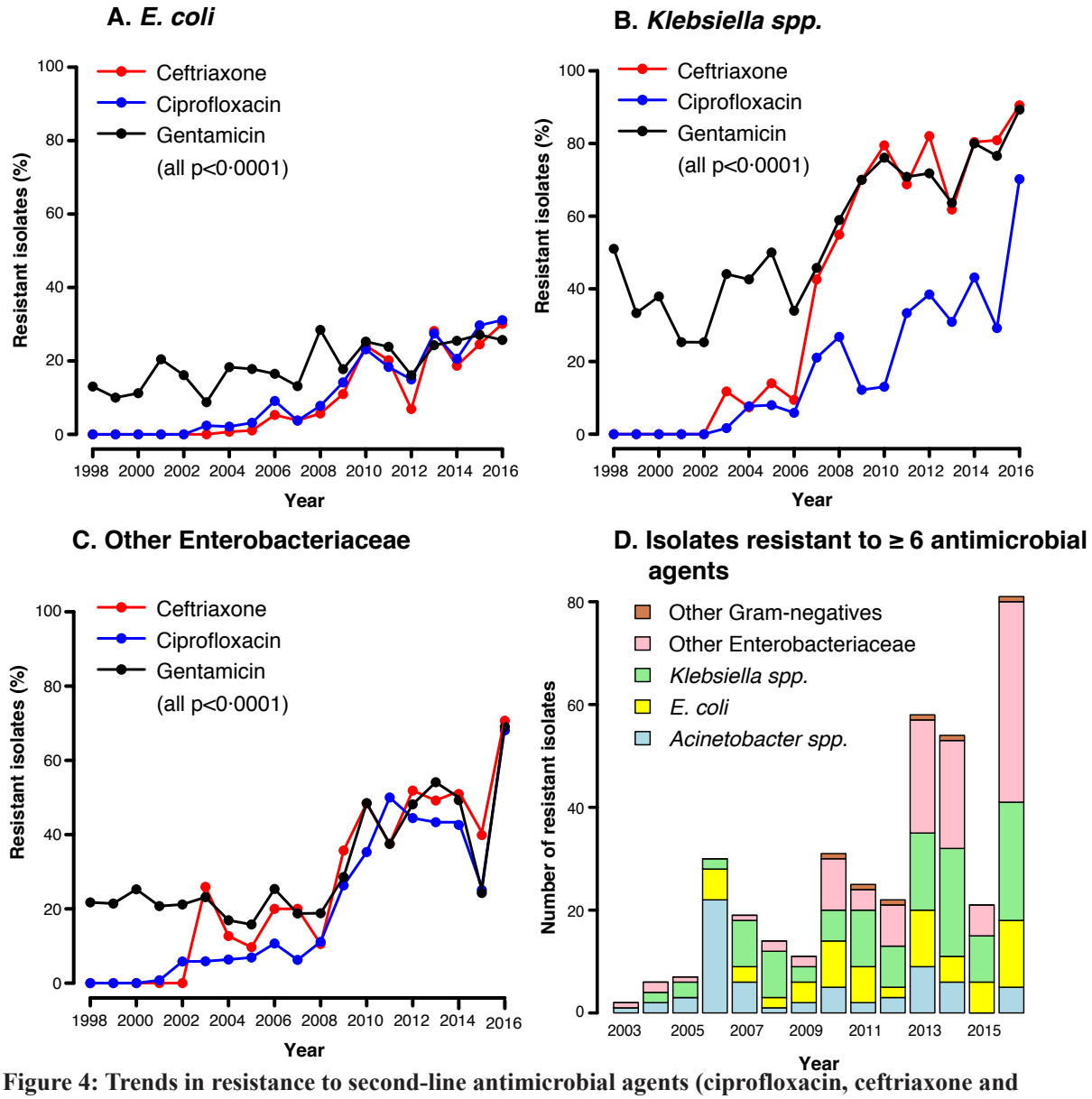


**Figure 4: Trends in resistance to second-line antimicrobial agents (ciprofloxacin, ceftriaxone and gentamicin)** in (A) *E. coli,* (B) *Klebsiella spp.* and (C) other Enterobacteriaceae *a*nd (D) trends in frequency of isolates resistant to all six commonly used antimicrobial agents in Malawi (*ampicillin, chloramphenicol, cotrimoxazole, ceftriaxone, ciprofloxacin and gentamicin).

SUPPLEMENTARY APPENDIX

# Trends in antimicrobial resistance in bloodstream infection isolates at a large urban hospital in Malawi (1998-2016): a surveillance study

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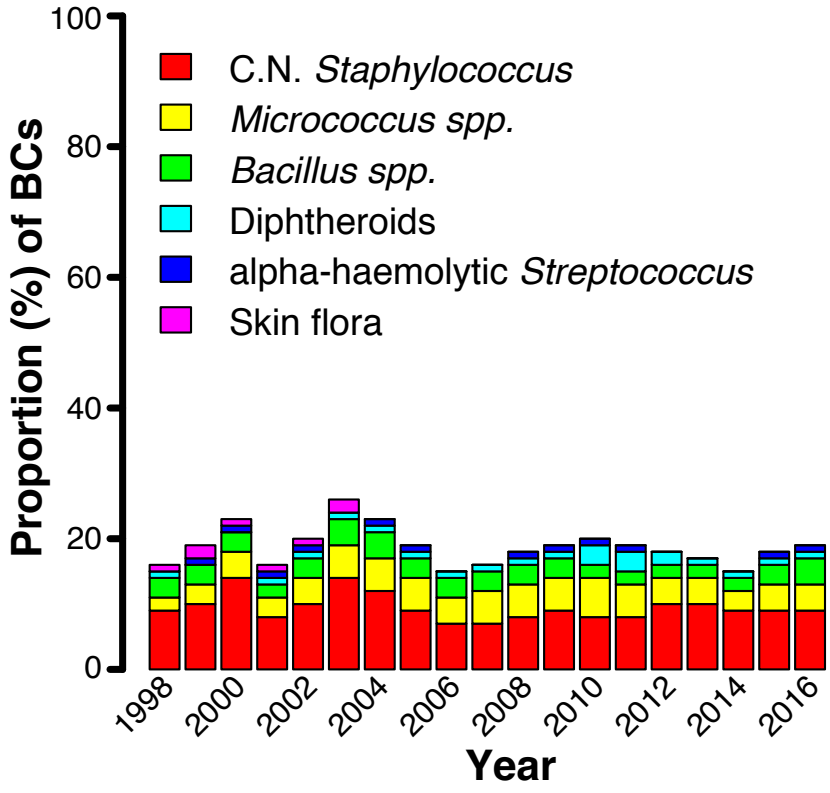
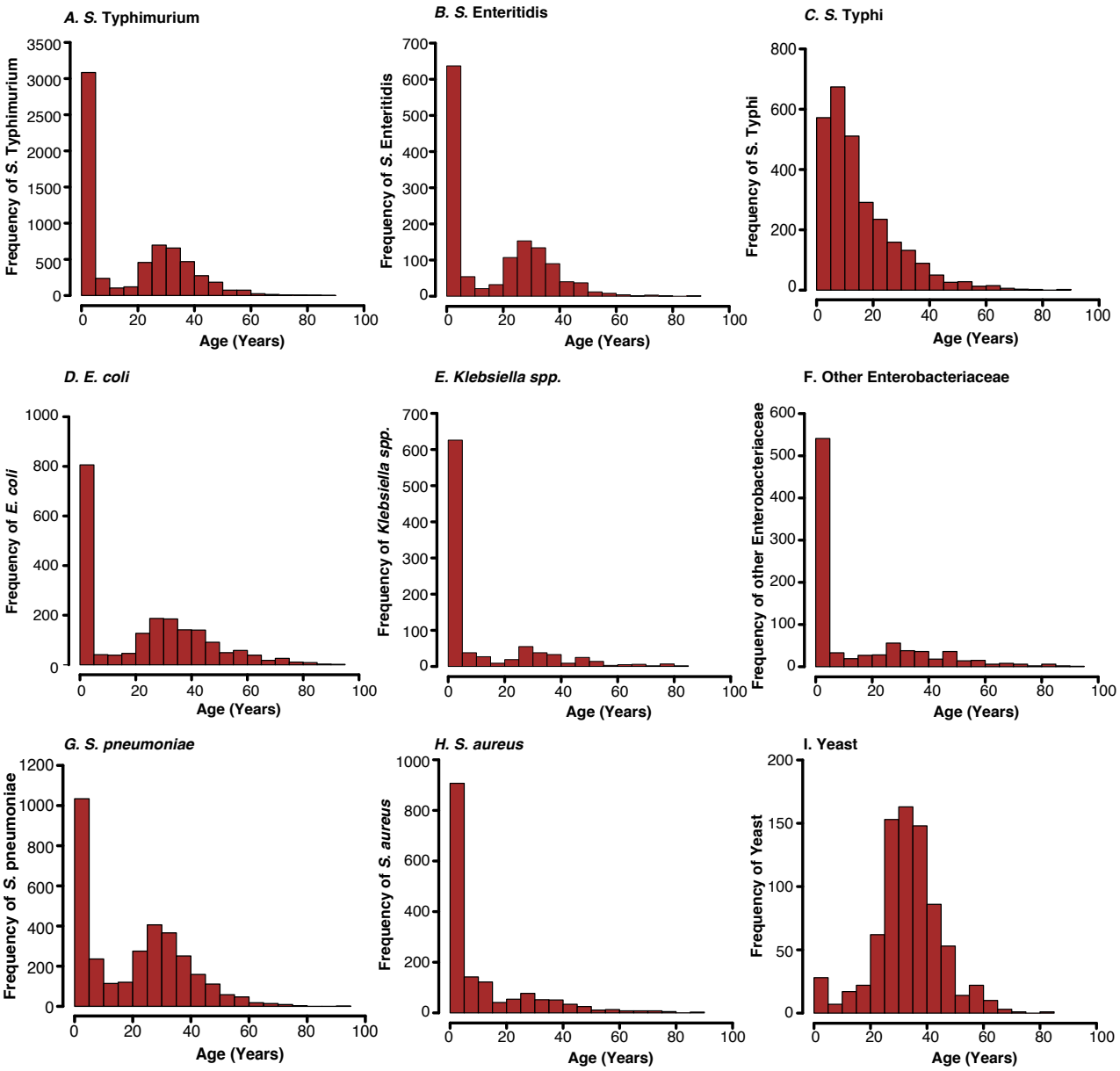
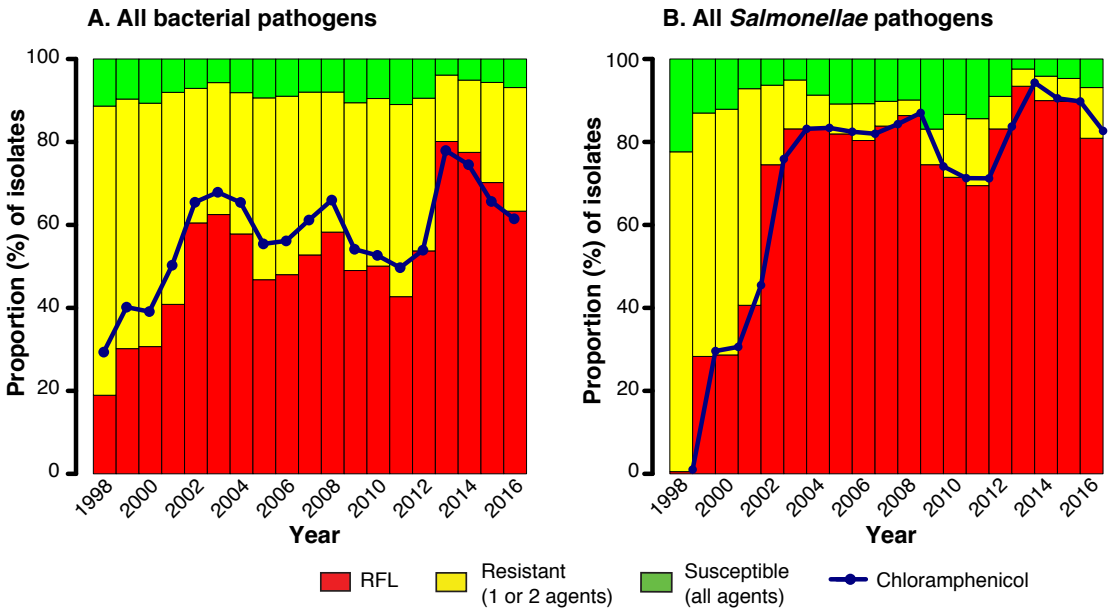
## SUPPLEMENTARY FIGURES



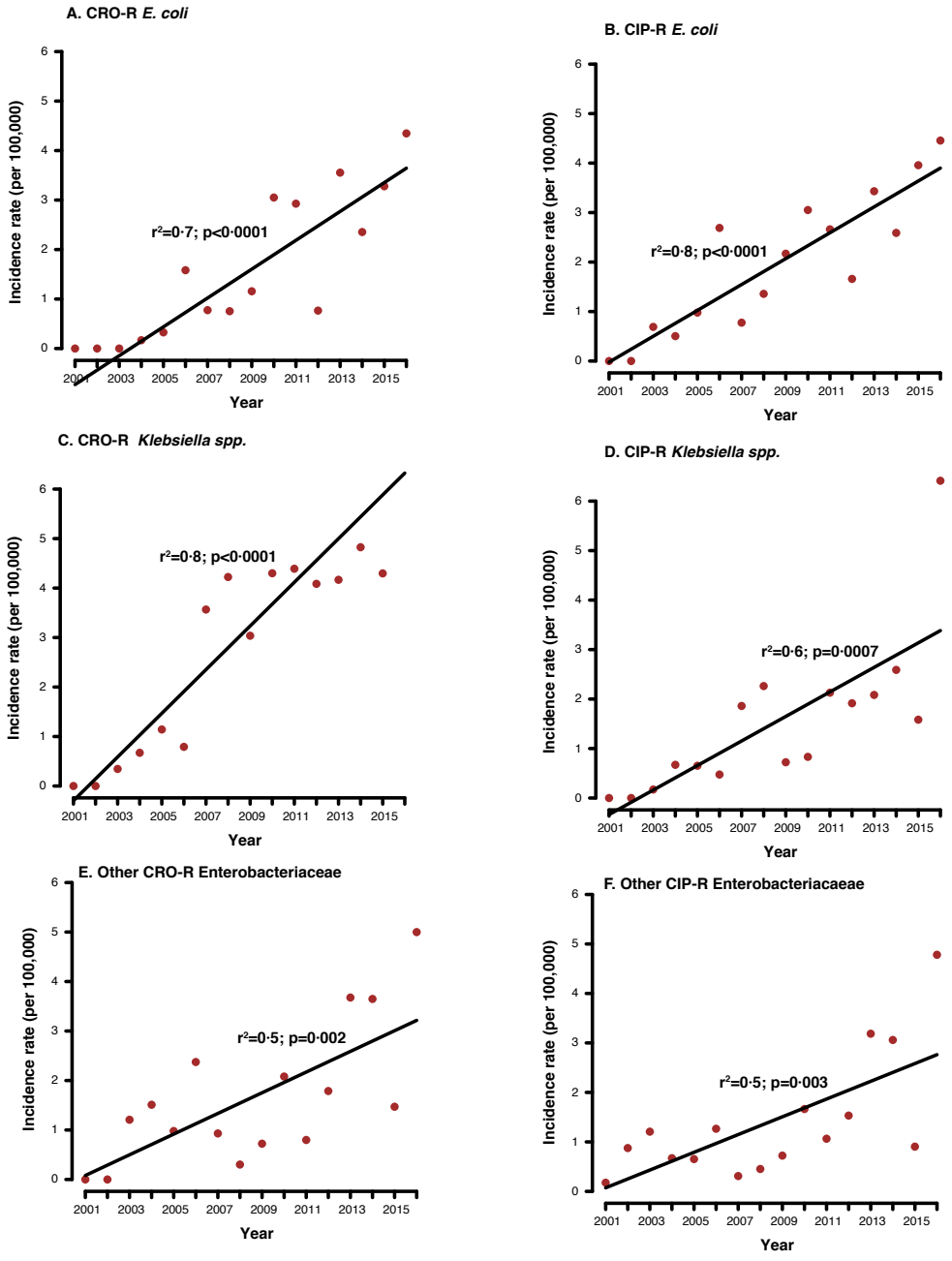

**Supplementary Figure 1:** Distribution of contaminants as proportions of all BCs by year of isolation.




**Supplementary Figure 2:** Age distributions of patients presenting to QECH between 1998 and 2014 with confirmed BSI caused by (A) *S.* Typhimurium, (B) *S.* Enteritidis, (C) *S.* Typhi, (D) *E. coli*, (E) *Klebsiella spp*., (F) other Enterobacteriaceae, (G) *S. pneumoniae*, (H) *S. aureus* and (I) yeast.

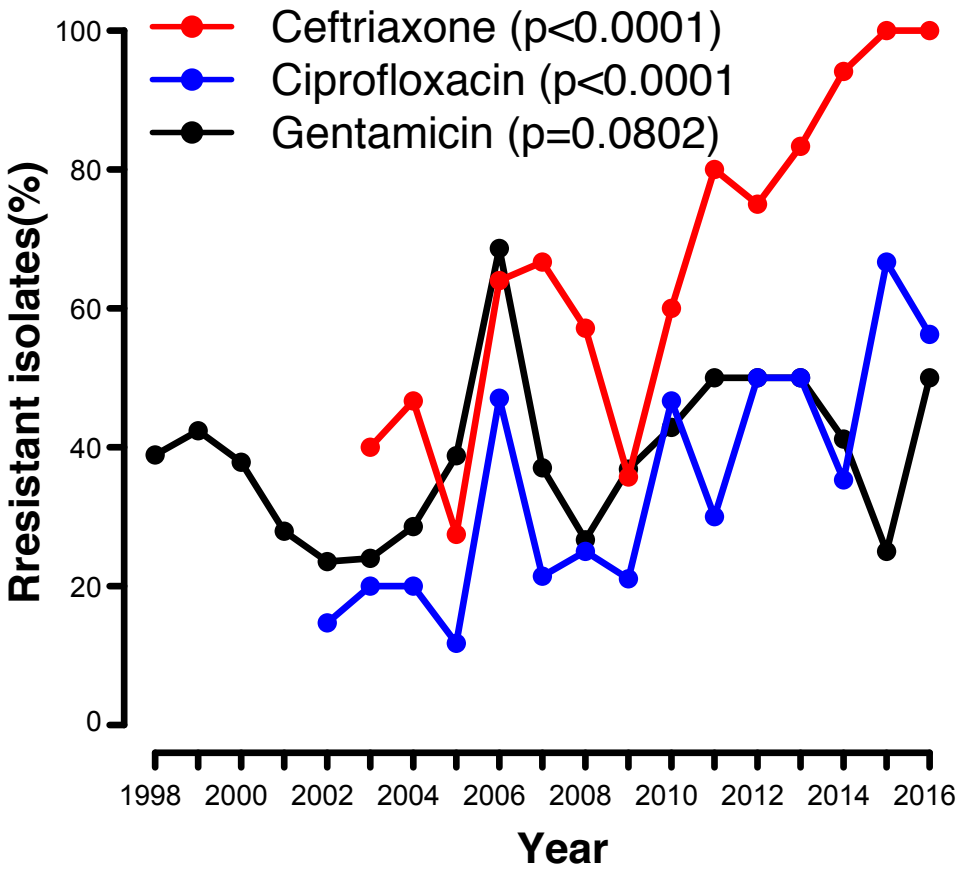



**Supplementary Figure 3**: Trends in resistance to first-line agents amongst in (A) all bacterial pathogens and (B) all *Salmonellae* isolates**.** As with Figure 3**,** first line antimicrobials include chloramphenicol and cotrimoxazole, plus ampicillin for Gram-negative pathogens and penicillin for gram positive pathogens.




**Supplementary Figure 4:** Trends in annual incidence (per 100,000) of Enterobacteriaceae resistant to ceftriaxone (CRO-R) or ciprofloxacin (CIP-R). A. Annual incidence of CRO-R *E. coli*; B. Annual incidence of CIP-R *E. coli*; C. Annual incidence of CRO-R *Klebsiella spp*; Annual incidence of CIP-R *Klebsiella spp.;* E. Annual incidence of Other CRO-R Enterobacteriaceae; F. Annual incidence of other CIP-R Enterobacteriaceae. In For each panel, points represent observed annual incidence rates while the black line graph represent annual incidence rates

predicted by a linear regression model with the general form $\boldsymbol{y} = \boldsymbol{\beta_0} + \boldsymbol{\beta_1 x}$, where $\boldsymbol{y}$ is the incidence rate, $\boldsymbol{x}$ is the year isolation and $\boldsymbol{\beta_0}$ and $\boldsymbol{\beta_1}$ are constant coefficients.




**Supplementary Figure 5:** Trends in proportions of *Acinetobacter spp.* isolates resistant to ceftriaxone, ciprofloxacin and gentamicin during 1998-2016.